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(±)8-Amino-5,6,7,8-tetrahydroisoquinolines as novel antinociceptive agents

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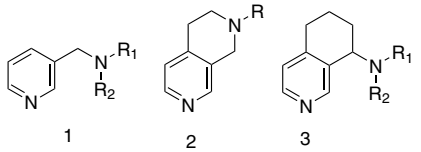
Abstract—Several amine-substituted 8-amino-5,6,7,8-tetrahydroisoquinolines were examined as conformationally-constrained analogs of the nicotinic cholinergic (nACh) 3-(aminomethyl)pyridines. Although these ligands failed to bind at nACh receptors, the *N*-ethyl-*N*-methyl analog **3d** was found to be at least equipotent with nicotine in rodent tests of antinociception. The mechanism of action of **3d** is currently unknown.

Effective pain management is one of today's most serious unmet problems. Agents that bind at nicotinic acetylcholinergic (nACh) receptors offer a relatively novel and unexploited mechanistic approach to the treatment of pain. In the course of our studies we have investigated a variety of nACh receptor ligands including conformationally-constrained ligands (reviewed[1,2]). For example, *N*-substituted 3-aminomethylpyridines such as **1** retain affinity for α4β2 nACh receptors and possess antinociceptive properties.[3] Although substitution at the pyridine 4-position has a tendency to reduce nACh receptor affinity,[3] compounds such as **2**, which might be viewed as conformationally-constrained analogs of **1**, bind at nACh receptors and display antinociceptive character.[4]



Because the most pharmacologically relevant conformation of nicotinic agents remains to be determined (but see Ref. 5), we have explored other analogs of the aminomethylpyridines where the side chain has been constrained. In the present investigation, we prepared a series of 8-aminotetrahydroisoquinolines (i.e., **3**) as conformationally-constrained analogs of **1** in order to determine their nACh receptor affinities and examine their antinociceptive properties. In general, aryl-unsubstituted analogs of **1** display low affinity ($K_i > 500$ nM) for α4β2 nACh receptors when the amine is a primary or secondary amine, or a tertiary amine where $R_1 = Me$ and $R_2 = n$-Pr; conversely, affinity is optimal ($K_i \leqslant 30$ nM) when $R_1 = Me$ and $R_2 = Et$, or where the amine is a pyrrolidine ring.[1,3] Similar amine-substituted derivatives of **3** were prepared for comparison.

Aminotetrahydroisoquinolines prepared for this investigation (Scheme 1) are shown in Table 1. Oxidation of 5,6,7,8-tetrahydroisoquinoline (**4**) with $KMnO_4$ provided the known[6] 5,6-dihydro-7*H*-isoquinolin-8-one (**5**), which was used as a common synthetic intermediate. Compound **5** was reductively aminated using sodium cyanoborohydride and the appropriate amine (method A) to afford target **3**. In method B, a methanolic solution of **5** was allowed to react with the $MeNH_2$, under catalytic hydrogenation conditions to reduce the intermediate imine, to afford **3b**; **3b** was acylated with AcCl and the corresponding amide **6** was reduced to **3d** with

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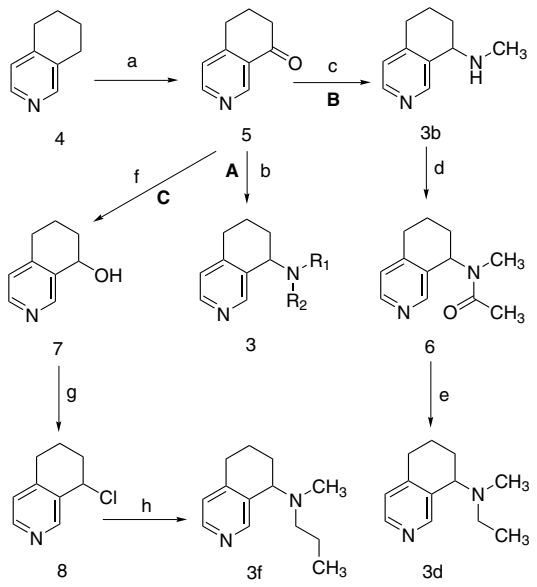


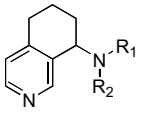
Scheme 1. Reagents and conditions: (a) $KMnO_4$, (b) R_1R_2NH, $NaCNBH_4$, (c) $MeNH_2$, H_2, (d) AcCl, (e) $LiAlH_4$, (f) $NaBH_4$, (g) $SOCl_2$, (h) HNR_1R_2.

$LiAlH_4$. An alternative role of synthesis (method C) involved $NaBH_4$ reduction of **5** to its corresponding alcohol **7**, reaction of **7** with $SOCl_2$ to afford the chloro compound **8**, and alkylation of **8** with *N*-methyl-*N*-propylamine to afford **3f**. Where a target compound was prepared by more than one method, thin-layer chromatography and [^{1}H]NMR were used to support structural identity.

In general, none of the compounds displayed appreciable affinity for α4β2 nACh receptors with the exception of **3c** ($K_i = 330$ nM) and **3d** ($K_i = 930$ nM) (Table 2). Compound **3c** binds at α4β2 nACh receptors with an affinity comparable to that of aminomethylpyridine **1** where $R_1 = R_2 = Me$ ($K_i = 540$ nM).[3] However, **3d** possessed >30-fold lower affinity than its ring-open analog (i.e., **1** $R_1 = Me$, $R_2 = Et$; $K_i = 28$ nM), and **3g** binds with >300-fold lower affinity than its corresponding aminomethylpyridine.[3] On this basis it seems unlikely that structures **3** represent pharmacologically-active conformations of **1** for interaction with α4β2 nACh receptors.

Even though compounds **3** lacked appreciable affinity for α4β2 nACh receptors, several were examined for

Table 1. Physicochemical properties of the aminotetrahydroisoquinolines



	R_1	R_2	Method[a]	Mp (°C)	%Yield	Recryst. solvent	Empirical formula[b]
3a	H	H	A	158–160	55	MeOH	$C_9H_{12}N_2 \cdot 1.5C_2H_2O_4$
3b	H	Me	B	193–197	47	2-PrOH/EtOH	$C_{10}H_{14}N_2 \cdot 1.8C_2H_2O_4$
			C	189–193	60	2-PrOH/EtOH	
3c	Me	Me	A	123–126	17	2-PrOH/MeOH	$C_{11}H_{16}N_2 \cdot 2C_2H_2O_4$
			C	203–205	45	MeOH/Et_2O	$C_{11}H_{16}N_2 \cdot 2HCl$
3d	Me	Et	B	143–146	38	2-PrOH/MeOH	$C_{12}H_{18}N_2 \cdot 2C_2H_4O_4$
			C	145–148	70	2-PrOH/EtOH	
3e	H	*n*-Pr	A	175–178	8	MeOH/Et_2O	$C_{12}H_{18}N_2 \cdot 1.5C_2H_2O_4$
3f	Me	*n*-Pr	A	98–100	15	MeOH/Et_2O	$C_{13}H_{20}N_2 \cdot C_2H_2O_4 \cdot 0.5MeOH$[c]
3g	$CH_2CH_2CH_2CH_2$		C	135–138	73	2-PrOH/EtOH	$C_{13}H_{18}N_2 \cdot 1.6C_2H_2O_4$

[a] See Scheme 1.
[b] Compounds analyzed within 0.4% of theory for C, H, N. Compounds **3b–d** were prepared by more than one route in an attempt to improve yields and explore the general applicability of the reaction scheme.
[c] Compound **3f** ($C_{13}H_{20}N_2$) also analyzed correctly for C, H, N as its free base (bp 90 °C/0.03 mmHg).

Table 2. Pharmacological properties of the aminotetrahydroisoquinolines **3**

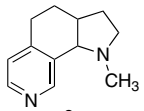
	K_i (nM) (SEM)	Tail-flick assay (ED_{50}, mg/kg) (subcutaneous)[a,b]	Tail-flick assay (ED_{50}, μg/mouse) (intrathecal)[a,c]	Hot-plate assay (ED_{50}, mg/kg) (subcutaneous)[a,b]
3a	>10,000	1%	NT	10%
3b	>10,000	0%	13 (10–17)	2%
3c	330 (36)	2%	8%	3.9 (1.4–11)
3d	930 (120)	0.6 (0.1–1.1)	12 (9–17)	0.2 (0.1–0.3)
3e	>10,000	NT	NT	NT
3f	>10,000	NT	NT	NT
3g	>10,000	2%	6%	11%
Nicotine	1.2 (0.4)	1.5 (0.8–2.2)	12 (8–16)	0.5 (0.3–0.7)

[a] Assays were performed as previously reported.[8] ED_{50} values are followed in parenthesis by (S.E.M.). NT = not tested.
[b] Where antinociception was not observed, %MPE is reported for a 10 mg/kg dose.
[c] Where antinociception was not observed, %MPE is reported for a 20–25 μg dose.

antinociceptive action in the mouse tail-flick assay (Table 2). Interestingly, compound **3d** was found to be twice as potent as (−)-nicotine. The antinociceptive actions of **3d** following subcutaneous administration were evident for 120 min, relative to 30 min for nicotine (data not shown). In addition, the antinociceptive effects, unlike those of nicotine, were not antagonized by the non-competitive nACh antagonist mecamylamine (1 mg/kg) nor the competitive antagonist dihydro-β-erythroidine (1 mg/kg) (data not shown). Similar results were obtained in the mouse hot-plate assay. Administered via the intrathecal route, **3d** was equipotent with (−)-nicotine in the tail-flick assay. In contrast, compounds **3a** and **3b** were inactive both in the tail-flick and hot-plate assays, whereas **3b** was nearly equipotent with **3d** and nicotine when administered via the intrathecal route. Compound **3c** was inactive in the tail-flick assay and nearly 20 times less potent than **3d** in the hot-plate assay. In summary, several of the compounds displayed antinociceptive action, but only **3d** was active under all three assay conditions—and was at least equipotent with (−)-nicotine in these assays.

With its low affinity for α4β2 nACh receptors, and the inability to antagonize its effects with nACh antagonists, it is difficult to reconcile the actions of **3d** with a nicotinic receptor mechanism unless there is involvement of a nACh receptor subtype other than α4β2 receptors. Compound **3d** was evaluated at several such subtypes and found to show little affinity: α2β2 ($K_i = 10,200 \pm 1700$ nM), α2β4 ($K_i = 49,900 \pm 4800$ nM), α3β2 ($K_i = 4410 \pm 730$ nM), α3β4 ($K_i = 46,900 \pm 3200$ nM), α4β2 ($K_i = 5860 \pm 880$ nM), α4β4 ($K_i = 40,000 \pm 1600$ nM), α6β4 ($K_i = 49,400 \pm 4100$ nM), α7 ($K_i = 10,500 \pm 200$ nM). Of course, the possibility exists that **3d** might still be acting via a subtype that was not specifically examined. However, because α4β2 receptors represent a major population of nACh receptors in mammalian brain, it seems unlikely that **3d** produces its antinociceptive effects via such a mechanism. Compound **3d** was also examined at 75 other neurotransmitter receptors (CEREP) and displayed remarkably low affinity for each; for example: $K_i > 10,000$ nM for hA$_1$, hA$_2$, and hA$_3$ adenosine receptors, α$_1$-, α$_2$-, hβ$_1$-, hβ$_2$-adrenergic receptors, central and peripheral BZ receptors, hD$_1$–D$_5$ dopamine receptors, H$_1$ and H$_2$ histamine receptors, hm$_1$–m$_5$ muscarinic cholinergic receptors, μ-, δ-, and κ-opioid receptors, h5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_3$, 5-HT$_{5A}$, 5-HT$_6$, and 5-HT$_7$ serotonin receptors, nor did it bind at the norepinephrine or dopamine transporter, or to K^+, Na^+, Ca^{++}, or Cl^- ion channels.

Compounds **9**[6] and **10**[7] are other examples of previously reported conformationally-constrained nicotine analogs. Both lack significant affinity for α4β2 nACh receptors yet possess antinociceptive character. Whereas the antinociceptive action of **10** was attenuated by mecamylamine,[7] that of **9**,[6] like that of **3d**, was not. The aminotetrahydroisoquinoline moiety common to **3d** and **9** and might be responsible for their antinociceptive properties.



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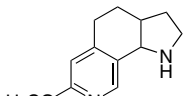

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In summary, with respect to interaction at α4β2 nACh receptors, it is quite doubtful that the aminotetrahydroisoquinolines **3**, represent pharmacologically important conformations of **1** due to their low affinity. Nevertheless, compound **3d** in particular displayed antinociceptive actions both in the tail-flick and hot-plate assays and, as such, might represent an interesting structural and mechanistic template for further development of novel analgesic agents.

Acknowledgements

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%MPE = [(test-control)/(10-control)] × 100. Groups of 6–12 mice were used for each dose, and mice were tested 5 min after either subcutaneous or intrathecal injections. Intrathecal injections were performed free-hand between the L5 and L6 lumbar space in unanesthetized mice according to the method of Hylden and Wilcox[9] using a 30-gauge needle attached to a glass microsyringe. The injection volume in all cases was 5 μL. In the hot-plate test, mice were placed into a 10 cm wide glass cylinder on a hot plate (Thermojust Apparatus) maintained at 55 °C, and the antinociceptive response was calculated as %MPE. Groups of 6–12 mice were used for each dose, and mice were tested 5 min after subcutaneous injections.

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